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Hybridization between escaped domestic and wild American mink (*Neovison vison*)

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Abstract

The release of domesticated organisms into natural populations may adversely affect these populations through predation, resource competition, and the introduction of disease. Additionally, the potential for hybridization between wild and domestic conspecifics is of great concern because it can alter the evolutionary integrity of the affected populations. Wild American mink (*Neovison vison*) populations may be threatened not only by competition for resources with domestic mink originating from farms, but by breeding with such escapees. Using 10 microsatellite loci, we genotyped mink from Ontario, Canada, sampled from two farms, two putatively mixed populations in regions surrounding the mink farms, and two wild populations with no recent history of mink farming. Using individual-based Bayesian population assignment, we identified four population clusters, including one wild, and three domestic populations. The latter were not clustered by farm but rather by distinct line-bred colour phases. Population clustering also identified domestic and hybrid mink in the free-ranging populations. Nearly two-thirds of the mink sampled in the two putatively mixed populations (78% and 43%) were either farm escapees or descendants of escapees. Principal components analysis of allele frequencies supported our Bayesian assignment results. The power of our assignment test was assessed using simulated hybrid genotypes which suggested that our overall correct classification rate was 96.2%. The overwhelming presence of domestic animals and their hybridization with mink in natural populations is of great concern for the future sustainability of wild mink populations.

Keywords: domestic, farm, microsatellite, mink, mink ranch, *Neovison vison*

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Introduction

The release from captivity of domesticated organisms is widely considered to be a threat to native biodiversity and the integrity of natural communities (Rhymer & Simberloff 1996; Garant *et al*. 2003; McGinnity *et al*. 2003). Natural populations can be negatively affected by domesticated organisms through predation, resource competition, and disease introduction (Manchester & Bullock 2000). However, when domesticated species have wild conspecifics, one of the most detrimental impacts is the infusion of domestic genes via interbreeding (Rhymer & Simberloff 1996; McGinnity *et al*. 2003). Interbreeding may introduce genes favoured under artificial selection that are maladaptive in the natural environment, and disrupt locally adapted gene complexes. Both of these processes can reduce the fitness of wild populations (Rhymer & Simberloff 1996; Allendorf *et al*. 2001; McGinnity *et al*. 2003; Hutchings & Fraser 2008). These issues are not trivial — the impact of introductions and farming on wild environments and their potential contributions to the loss of biological diversity is among the 100 major ecological concerns of interest to policy makers in the UK (Sutherland *et al*. 2006). Interbreeding between domesticated and wild counterparts has been observed in terrestrial carnivores, ungulates, fowl, anurans and many fish species (Rhymer & Simberloff 1996; Williams *et al*. 2002). Interbreeding with domesticated conspecifics may alter the evolutionary integrity (Dobzhansky 1970) of the infused wild populations (Williams *et al*. 2002; Latch *et al*. 2006; Hutchings & Fraser 2008; Randi 2008).

Domesticated populations have low genetic variation because they are subjected to intense directional selection and line breeding as well as relaxed natural selection (Price

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1984; Belliveau *et al*. 1999). Agricultural breeding programmes actively select for high production as well as for behaviour and morphological characteristics desired by humans; the selection of such characteristics may cause deleterious genes and phenotypes to increase or become fixed (Price 1984; Rauw *et al*. 1998; Lynch & O'Hely 2001). Similarly, food supplementation, lack of competitive mate selection, and the reduction or absence of predation risks in captivity all result in a relaxation of selection that may cause deleterious genes and phenotypes to increase in frequency (Price 1984; Snyder *et al*. 1996; Belliveau *et al*. 1999). Furthermore, unintentional and drag-along selection is common not only through the environment but through linked traits (Price 1984; Snyder *et al*. 1996). Thus, active selection and relaxed natural selection in captivity promote an increase in the frequency of alleles that may be deleterious in nature (Allendorf *et al*. 2001; Lynch & O'Hely 2001; Randi 2008). Domestic alleles introduced into the wild may even result in fitness reductions such that natural populations are incapable of sustaining themselves (Lynch & O'Hely 2001).

Perhaps the most profound and best-studied examples of the introduction of domesticated organisms into the natural environment are the introductions of farmed fish, especially salmonids (*Salmo* spp., Hutchings & Fraser 2008). The introduction of farmed salmonids to natural populations has resulted not only in resource competition with their wild counterparts but also in 'hybridization' between farmed and wild animals (Fleming & Einum 1997; McGinnity *et al*. 2003; Hutchings & Fraser 2008). The list of observed consequences of farm-wild hybridization is extensive and includes reduced survival and fitness of the F_1 and F_2 generations, accelerated growth rate, decreased predator avoidance behaviours and increased agonistic behaviours (McGinnity *et al*. 2003; Wessel *et al*. 2006; Hutchings & Fraser 2008). McGinnity *et al*. (2003) found that hybrid juveniles displaced wild parr individuals, despite hybrids having reduced survival and outbreeding depression. Furthermore, McGinnity *et al*. (2003) linked repeat introductions (i.e. escapes) to a reduced likelihood of native population persistence, because of continuous opportunity for hybridization and introgression causing cumulative fitness depression (McGinnity *et al*. 2003). Araki *et al*. (2008) concluded that rapid fitness declines in wild salmonid populations could be achieved with strong selection on a single trait in both the captive and wild environment. However, rapid declines in fitness may also be induced when artificial selection acts on multiple traits throughout the life cycle, such as growth, foraging and reproduction (Araki *et al*. 2008).

In addition to the well-studied plight of wild salmon, concerns surrounding hybridization between domesticated animals and their wild progenitors have been raised for several terrestrial carnivores including the gray wolf (*Canus lupus*), Scottish wild cat (*Felis silvestris grampia*), European wild cat (*Felis silvestris*), polecat (*Mustela putorius*), and Arctic fox (*Alopex lagopus*) (Norén *et al*. 2005; Randi 2008). Another wild carnivore with a domestic counterpart is the American mink (*Neovison vison*), a semi-aquatic mustelid endemic to North America that has been trapped for its fur for centuries (Joergensen 1985). Owing to high demand for fur, the trapping of wild mink was supplemented by the farming of mink beginning in the late 1800s (Joergensen 1985; Belliveau *et al*. 1999; Kruska & Sidorovich 2003). Domesticated mink have been bred intensively for artificially selected traits including fur colour, size and temperament (Belliveau *et al*. 1999; Kruska & Sidorovich 2003). Mink have been bred to exhibit a broad range of colours, from snow white to jet black with the individual colour types being referred to as colour phases (Shackelford 1948; Joergensen 1985). Many of the colour phases are exclusively line bred because the colour is recessive to the standard brown, whereas others are blended. For example, mahogany is achieved by breeding the black and standard brown phases together (Joergensen 1985). Artificial selection has not been restricted to physical attributes. Fearfulness and fear-induced aggression in mink may be beneficial in a natural context but may be detrimental to the animal's welfare in the captive environment and this was therefore one of the first traits selected against in the history of mink domestication (Trapezov 2000; Malmkvist & Hansen 2002).

Through accidental escapes and deliberate releases caused primarily by animal rights activists, domestic mink have become established in regions outside their native range; in other words, they have become 'feral' (Joergensen 1985; Lodé *et al*. 2001; Kruska & Sidorovich 2003; Reynolds *et al*. 2004; McDonald *et al*. 2007). These feral, invasive mink have contributed to declines of local endemic mustelids through competition, and to declines of many birds, voles, amphibians, and invertebrates through predation in Europe, Iceland and South America (Medina 1997; Lodé *et al*. 2001; Reynolds *et al*. 2004; Bonesi & Palazon 2007). Several studies have been conducted on the ecological impacts of feral mink in regions where they are not native, such as Europe (Lodé *et al*. 2001; Reynolds *et al*. 2004). However, the fur farming industry is also active within the native range of American mink, and little is known about what effects domestic mink might have on their wild counterparts. Recently, Bowman *et al*. (2007) examined the relationship between the wild mink harvest and mink farm density in Canada. They found a positive relationship between mink farm density and density of trapped mink per province, which have both been in decline in recent years. This study described a phenomenon whereby up to 38% of mink trapped per province per year from free-ranging populations were evaluated by fur graders at auction as being of domestic origin. These results strongly suggest that domestic mink are being caught by mink trappers and

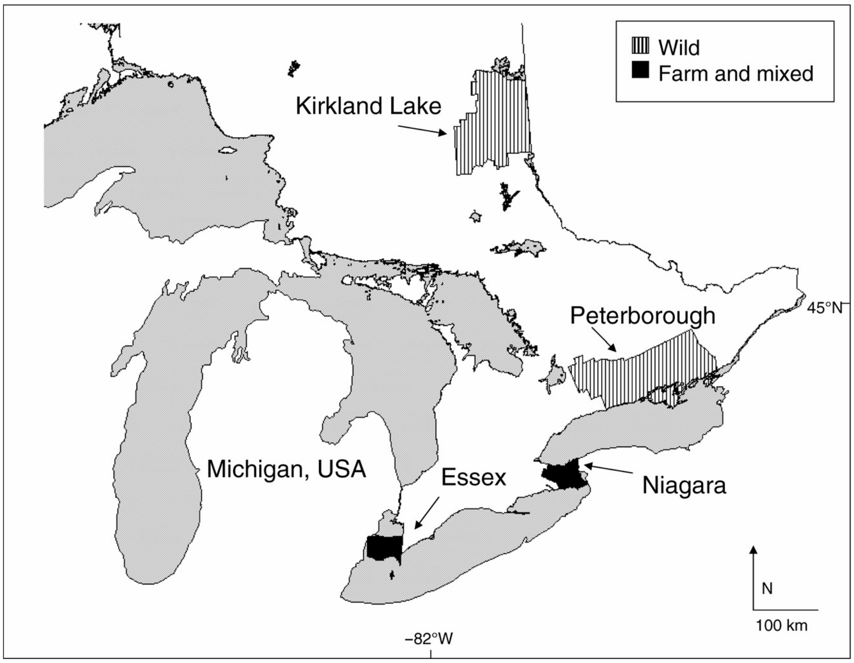

Fig. 1 Locations in Ontario, Canada where American mink (*Neovison vison*) were sampled in the wild and in mink farms during 2005–2006. Included are two wild populations (wild 1 from Kirkland Lake and wild 2 from Peterborough), the overlapping farms and surrounding putatively mixed populations (mixed 1 and farm 1 in Essex County; mixed 2 and farm 2 in the Municipality of Niagara).

that abundant feral animals may be contributing to a decline in wild American mink populations, perhaps via outbreeding depression or disease introduction (Bowman *et al*. 2007).

We tested the hypothesis that, as with regions of the world where mink are farmed but not endemic, farm-raised mink escape (or are released) and persist in the wild environment where American mink are endemic (e.g. Bowman *et al*. 2007). Furthermore, we hypothesized that these domestic mink would breed with the wild mink and produce domestic–wild hybrids (hereafter referred to as hybrids). We collected tissue samples from six mink populations in four regions (Fig. 1) that represented wild populations, domestic populations, and putatively mixed free-ranging populations that were adjacent to the farms. We used microsatellite DNA loci to examine mink population structure and to assign individuals to inferred populations. We predicted that we would identify not only domestic mink living in the natural populations adjacent to mink farms but also domestic–wild hybrids.

Table 1 Summary data for American mink (*Neovison vison*) sampled in the wild and in mink farms during 2005–2006 in Ontario, Canada, and genotyped at 10 microsatellite loci. Depicted is each geographical grouping and colour phase including the number sampled from each population, the number of loci typed, the mean number of alleles (N_a), observed heterozygosity (H_O) and F_{IS} for each as well as the unbiased estimates of Hardy–Weinberg equilibrium [exact *P* values by the Markov chain method (HWE *P*) for deficiency of heterozygotes]

Population	Region	N	N_a	H_O	F_{IS}	HWE P
Wild 1	Kirkland Lake	30	7.0	0.662	0.057	0.002
Wild 2	Peterborough	20	6.0	0.706	0.003	0.589
Mixed 1	Essex	21	6.6	0.615	0.170	0.000
Mixed 2	Niagara	32	7.7	0.708	0.091	0.000
Farm 1						
Black	Essex	29	5.8	0.651	0.045	0.101
Brown	Essex	28	6.2	0.703	0.025	0.514
Pastel	Essex	15	5.4	0.621	0.110	0.005
Farm 2						
Iris	Niagara	20	5.1	0.622	0.037	0.013
Mahogany	Niagara	20	5.9	0.604	0.175	0.000

Methods

Sample collection

Six populations of American mink were sampled in 2005 and 2006; two each of domestic, 'putatively wild', and 'putatively mixed' (Fig. 1, Table 1). Sampling took place within the Great Lakes watershed in Ontario, Canada, which has historically been a region with abundant wild American mink (Eagle & Whitman 1987). Putatively mixed populations were free-ranging animals in regions containing mink farms that we predicted would be composed of both wild and domestic mink and their hybrids. Putatively wild mink were sampled by obtaining trapped carcasses during winter 2005–2006 from trappers in both the Kirkland Lake (13 698 km^2, hereafter referred to as wild 1) and Peterborough (15 381 km^2, hereafter referred to as wild 2) administrative districts of the Ontario Ministry of Natural

Table 2 Mink primers used in genotyping analysis of American mink (*Neovison vison*) sampled in the wild and in mink farms during 2005–2006 in Ontario, Canada. Included are range of allele size, number of alleles, fluorescent primer label, primer-specific annealing temperature (T_a) concentration of primer used in each 10-μL reaction, observed (H_O) and expected heterozygosities (H_E), F_{IS} with bold indicating those loci that exhibited a significant deficiency of heterozygotes determined by the Markov chain method

Primer	Range	Alleles	Label	T_a(°C)	Primer (mM)	H_O	H_E	F_{IS}	Source
Mvi 2243	123–157	12	6-FAM	63.5	0.25	0.588	0.707	**0.128**	(Vincent *et al*. 2003)
Mvi 1016	218–236	10	6-FAM	63	0.25	0.730	0.782	**0.059**	(Farid *et al*. 2004)
Mvi 111	84–106	10	HEX	55	0.20	0.656	0.692	0.056	(O'Connell *et al*. 1996)
Mvi 1006	136–168	14	6-FAM	59.1	0.25	0.617	0.746	**0.168**	(Farid *et al*. 2004)
Mvi 099	324–356	15	6-FAM	60	0.15	0.767	0.771	0.008	(Fleming *et al*. 1999)
Mvi 1302	203–223	10	HEX	61	0.20	0.694	0.728	**0.058**	(Vincent *et al*. 2003)
Mvi 1321	88–116	12	6-FAM	63	0.15	0.679	0.739	**0.069**	(Vincent *et al*. 2003)
Mvi 4001	223–233	6	HEX	60	0.10	0.521	0.535	0.045	(Anistoroaei *et al*. 2006)
Mvi 1014	125–143	11	6-FAM	61	0.20	0.608	0.698	**0.145**	(Farid *et al*. 2004)
Mvi 114	62–82	9	HEX	61	0.30	0.685	0.727	**0.063**	(O'Connell *et al*. 1996)

Resources (OMNR). Wild 1 and wild 2 were separated by about 400 km. Both populations were predicted to be composed only of wild mink due to the absence of mink farms in these districts. We estimated that the closest mink farms to wild 1 and wild 2 were each at least 40 km distant. It was difficult to be certain about the presence and locations of mink farms in different regions however, due to the deregulation of the industry in the 1990s. Furthermore, mink farmers are justifiably protective of their locations and do not advertise such information primarily due to the activities of animal rights groups.

The two mink farms sampled were in the regions of Essex (1720 km^2, hereafter referred to as farm 1) and Niagara (1850 km^2, hereafter referred to as farm 2) in southern Ontario, Canada. Both of these mink farms have been in operation for > 20 years. We had no a priori knowledge of the quantity of mink escaping from these, or any other farms, so farms could not be stratified on this basis. Three colour phases were sampled from farm 1 and two colour phases from farm 2 (Table 1). The colour phases sampled included the genetically dominant 'standard brown,' the lighter brown 'pastel', the silver 'iris', 'black', and the black–brown hybrid 'mahogany'. The domestic mink were sampled by collecting carcasses from the farms when the furs were being harvested and therefore already sorted by colour phase.

The free-ranging populations in the regions adjacent to the sampled farms (hereafter mixed 1 and mixed 2) were sampled by live trapping during 2005 and 2006. Live trapping took place in the landscapes surrounding the farms, at a range of distances from the farms of 0 to 30 km. A second mink farm occurred within the landscape of our mixed 1 sampling, but not within mixed 2. Tissue samples collected from live trapped mink included blood obtained by clipping a nail and nicking the quick as well as hair samples collected by plucking hairs with roots attached.

Microsatellite genotyping

Whole DNA was extracted following the modified QIAGEN (QIAGEN) extraction protocol described by Guglich *et al*. (1994). DNA yield was quantified using a FLUOstar Optima fluorometer (BMG Labtechnologies) and Hoeschts Dye (Bio-Rad). Extracted DNA concentrations were calculated based on their relationship to the standard curve produced by the fluorescence of the calf thymus DNA. Calculated DNA concentrations were used to create standardized dilutions of 2.5 ng/μL (where yield was in excess of standard) for each sample, as a working dilution for polymerase chain reaction (PCR) amplification.

All samples were amplified using primers for 12 polymorphic microsatellite loci (of which two were dropped from analysis due to incomplete genotyping and the possible presence of null alleles — see Results) and pooled in four groups for genotyping (Table 2). Forward primers were fluorescently labelled with either 6-FAM or HEX (Integrated DNA Technologies) (Table 2). Amplifications were performed in 10-μL total volumes containing a final concentration of 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.1–0.3 mM forward and reverse primer depending on individual locus (Table 2), 5 ng DNA and 0.5 U of *Taq* polymerase.

Amplification was carried out with an initial denaturing period of 4 min at 94 °C followed by 30 cycles of 94 °C for 1 min, primer-specific annealing temperature (Table 2) for 30 s and 72 °C, followed by a final extension of 4 min at 72 °C. All amplifications were conducted alongside positive and negative controls in order to ensure the reliability of the reaction and its reagents. Mvi 1016 and Mvi 1321 were run for 30 s at 94 °C denaturing for each cycle, and Mvi 114 and Mvi 111 were run for 35 cycles.

All PCR-amplified products were precipitated in ethanol to remove excess salts and resuspended in 10-μL ddH_2O. A pooled dilution of 0.5 μL was then resuspended to 10 μL high HiDi formamide and ROX size standard 350 in a new plate and analysed with an ABI 3730 automated sequencer using GeneMapper 4.0 (Applied Biosystems) at the OMNR genetics laboratory at Trent University.

Analysis of genotypes

Only individuals that had at least 8 of 10 genotyped loci were used in subsequent analyses. We tested for the presence of null alleles at all loci and genotyping errors with Micro-Checker (van Oosterhout *et al.* 2004). Significant deviations from Hardy–Weinberg equilibrium (HWE) for both individual populations and globally for each locus were evaluated using an exact probability test in GenePop 4.0 (Rousset 2008). Allele frequencies, observed and expected heterozygosities, F_{IS}, linkage disequilibrium and pairwise population differentiation (F_{ST}) were analysed using FSTAT 2.9.3.2 updated from Goudet (1995).

Bayesian assignment tests (Structure 2.2 with the admixture model) were employed to evaluate the number of inferred genetic population clusters (K) and to assign individuals to their likely population of origin, without prior geographical information or an inferred population of origin (Pritchard *et al.* 2000; Falush *et al.* 2003). The number of inferred populations was assessed using the entire sample set ($n = 215$). Results were generated using five repetitions of 5×10^5 iterations following a burn-in period of 5×10^5 iterations. Burn-in length was selected based on the convergence in summary statistics in a series of trial runs. We selected the ideal K (population clusters) using the posterior probability of the data [Ln $P(D)$] by employing the formula [Ln $P(D)_k$ – Ln $P(D)_{k-1}$] as suggested by Garnier *et al.* (2004). Individuals were assigned probabilistically to populations or jointly to two or more if their genotypes indicated that they were admixed by their mean membership coefficient (q). Individuals were assigned to clusters with a minimum membership of $q \geq 0.80$ (this threshold was assessed using a simulation analysis described below) or jointly to two or more clusters such that the minimum sum of $q_i + q_j + ... q_n \geq 0.80$ for admixed individuals, thereby ensuring that at least 80% of an individual's genome was assigned to the inferred cluster(s) (Pierpaoli *et al.* 2003; Verardi *et al.* 2006). Furthermore, we were interested in domestic–wild hybrids, rather than domestic–domestic hybrids, since some of these latter animals may have resulted from breeding practices on farms (e.g. mahogany mink are black–brown hybrids). Therefore, where the minimum sum of $q_i + q_j + ... q_n \geq 0.80$ resulted only in domestic groups, we did not consider them hybrids, but rather assigned the individual to the group with the highest q. All mink assigned as hybrids contained some wild ancestry. Population differentiation (pairwise F_{ST}) was assessed using cluster assignments, including a hybrid cluster composed of individuals of mixed assignment. For this F_{ST} analysis, hybrids were identified from the mixed and wild groups, and later assigned to their own group (hybrid). Finally, we made no attempt to identify backcrossed individuals. This was not required to test our hypothesis (that hybridization was occurring), and we did not have adequate resolution in our markers to confidently identify backcrosses. We simply considered all animals with $q < 0.80$ as hybrids, recognizing that some of these individuals may have actually been backcrossed to domestic or wild groups. Similarly, some individuals with $q \geq 0.80$ to one group may have introgressed alleles from the other group due to repeated backcrossing. The net effects of this approach were likely to overestimate our classification success (see simulation analysis below) but also to underestimate the prevalence of admixed mink. We viewed this as conservative given our hypothesis test was to detect domestic–wild hybrids.

Supporting analyses

Fifty highly assigned ($q > 0.94$) individuals from each of the wild and farm clusters (where $K = 2$) were randomly chosen for modelling using HybridLab version 1.0 (Nielsen *et al.* 2006; Barilani *et al.* 2007). The genotypes of the selected mink were used to simulate 100 new individuals of each of wild and domestic mink as well as 35 domestic–wild hybrids. These genotypes were simulated using a random sampling of alleles based on their frequency in the parental population (Nielsen *et al.* 2006; Randi 2008). The samples sizes of each group were selected to approximate our real data set. Admixture analysis was again carried out using Structure 2.2 employing the same specifications used with the real data set, for $K = 2$. We compared classification success for different membership coefficients (q), and estimated mean (± 95% confidence intervals) wild membership coefficients for each of the three groups (wild, domestic, and hybrid).

Preliminary analyses suggested some loci had high values of F_{IS}, which may indicate a lack of HWE. Given that HWE is an assumption of our Bayesian assignment test, we sought to compare our findings to a second clustering technique that did not assume HWE. We conducted a principal components analysis (PCA) of allele frequencies (e.g. Patterson *et al.* 2006; Jombart 2008), comparing the PCA results to the Structure 2.2 results for $K = 2$. For each individual, we gave each allele a value of either 1 (homozygous), 0.5 (heterozygous), or 0 (absent). We then assessed the ability of the principal components to characterize the domestic–wild gradient. Finally, we compared factor scores for mink assigned using Structure to the three groups: wild, domestic, and hybrid. We used S-Plus 6.0 for the PCA (Insightful Corp.), and a covariance matrix with no rotation.

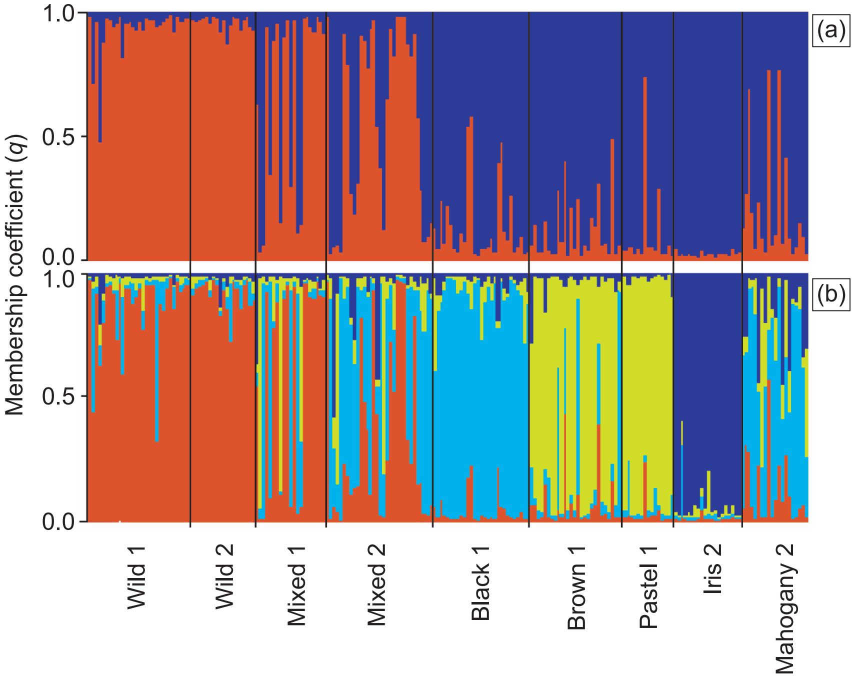

Fig. 2 Admixture analysis of American mink (*Neovison vison*) genotypes sampled in the wild and in mink farms during 2005–2006 in Ontario, Canada. Analysis was performed using Structure 2.2 for (a) $K = 2$ and (b) $K = 4$ clusters grouped by geographical origin, with each vertical bar representing the membership coefficient (q) for each individual. The analysis where $K = 2$ (a) differentiated between wild (red) and domestic (blue) genotypes. $K = 4$ (b) differentiated between wild (red) and domestic genotypes as well as genetic populations within the farms based on colour phase including black (light blue); brown (yellow) and iris (blue). Numerals after colours on X axis refer to sampling location in Ontario (Wild 1 = Kirkland Lake; Wild 2 = Peterborough; Mixed 1 = Point Pelee; Mixed 2 = Niagara; Black 1, Brown 1, and Pastel 1 = a farm in the Point Pelee area; and Iris 2 and Mahogany 2 = a farm in the Niagara area).

Results

Genetic variation and population diversity

Genotyped positive controls showed no sign of genotyping error (< 1%). Samples that did not amplify or genotype successfully on the first attempt were re-run to fill the missing genotypes. Analysis of genotyping data in Micro-Checker found no evidence of large allelic dropout or scoring error resulting from stuttering, but it identified 2 of 12 loci (Mvi 1010 and Mvi 4052) that may have shown signs of null alleles based on an homozygous excess. Furthermore, these two loci had a high proportion of missing genotypes and for these two reasons, they were omitted from our analyses. Individual genotypes were determined for 215 mink from the 10 remaining microsatellite loci. All loci were polymorphic with 6–15 alleles per locus (Table 2). The mean proportion of individuals successfully genotyped per locus was 90.4% (Table 2). No loci were in linkage disequilibrium at Bonferroni-corrected $P < 0.05$. A significant deviation from HWE was observed ($P < 0.01$), caused by a deficiency in heterozygotes globally. The groups were separated for population HWE analysis and several showed a deficiency of heterozygotes including wild 1, mixed 1, mixed 2, and all of the farm colour phases with the exception of brown and black (Table 1). F_{IS} values per population reflected this deficiency and indicated the presence of low to strong inbreeding with values ranging from 0.003 to 0.175 per population (Table 1).

Population assignment

The results of Bayesian analysis signalled two ($K = 2$) and four ($K = 4$) distinct populations within all the mink sampled (Fig. 2). The $K = 2$ analysis differentiated between wild and domestic mink genotypes (Fig. 2a). However, the same analysis signalled a second level of differentiation at four populations, which described several domestic populations, differentiated not by farm origin but by colour breeding line. The four populations described by $K = 4$ included one wild group and three domestic colour phases: black, brown (including both brown and pastel individuals) and iris (Table 3; Fig. 2b). The mahogany individuals were assigned as black–brown hybrids, which was consistent with the breeding of the mahogany colour phase (Table 3). We conducted further descriptive analysis using the $K = 4$ populations based on its capacity for greater resolution without a loss of confidence in the assignments. Four populations clearly distinguished between farmed and wild mink as well as among the basic domestic colour types (Fig. 2b).

Mean F_{ST} calculated using the 'F-model' for $K = 4$ populations estimated the divergence from the inferred common ancestor, indicating that the wild population was least diverged while the farm iris population was the most diverged from this inferred ancestor (Table 3) (Falush *et al.* 2003).

We ranked the individual q values to the wild population cluster for the 53 mink sampled in the putatively mixed

Table 3 Bayesian clustering analysis for 215 American mink (*Neovison vison*) sampled in the wild and in mink farms during 2005–2006 in Ontario, Canada. Analysis was performed using Structure 2.2 (Pritchard *et al.* 2000) including the membership coefficients (*q*) with the assigned cluster in bold as well as the given mean F_{ST} for each cluster that refers to the estimated drift from inferred common ancestor of all populations

	Cluster I	Cluster II	Cluster III	Cluster IV
Population of origin	Wild	Farm – Brown	Farm – Black	Farm – Iris
Wild 1	**0.861**	0.03	0.088	0.021
Wild 2	**0.919**	0.02	0.039	0.022
Mixed 1	0.613	0.147	0.204	0.037
Mixed 2	0.358	0.096	0.481	0.065
Farm 1 – Black	0.043	0.063	**0.858**	0.035
Farm 1 – Brown	0.068	**0.725**	0.172	0.036
Farm 1 – Pastel	0.032	**0.904**	0.044	0.02
Farm 2 – Iris	0.012	0.031	0.029	**0.929**
Farm 2 – Mahogany	0.126	**0.161**	**0.557**	0.156
Mean F_{ST}	**0.060**	**0.131**	**0.111**	**0.240**

populations and ordered them from lowest to highest where an individual with $q = 1$ would be a purely wild mink and $q = 0$ would be a purely domestic individual (Susnik *et al.* 2004) (Fig. 3). The 90% probability limits for *q* suggested that several individuals in the mixed populations could be confidently assigned to the wild ($q \geq 0.80$) or domestic ($q \leq 0.20$) group. However, since there was no 'hybrid' group in the analysis, we could not confidently assign hybrids to any one group. Thus, it was expected that hybrid individuals should have wide probability limits (Pritchard *et al.* 2000), and this is what we observed. We used the mean *q* values to the wild cluster to estimate the prevalence of hybrids. Hybridization was identified between the wild mink and all three of the farm colour phases, although the genetic contribution of the iris mink to the hybrids was much less than the black and brown phases, occurring in only 2 of 15 hybrids. Individuals sampled from mixed 1 included 57% wild individuals [7 females (f), 4 males (m) and 1 unknown sex], 10% hybrids (1 f, 1 m), and 33% domestic individuals (1 f, 6 m) (Fig. 3a). Mixed 2 included 22% wild individuals (2 f, 5 m), 41% hybrids (5 f, 8 m), and 38% domestic individuals (7 f, 2 m, and 3 unknown sex; Fig. 3b). In total, hybrid mink composed 28% of the mink sampled from the putatively mixed groups (mixed 1 and mixed 2), whereas 36% were escapees from local farms, and only 36% of the mink sampled from both populations were identified as wild mink. We further identified 1 of the 20 (5%) mink in wild 2 as a hybrid individual and 5 of the 30 (17%) mink sampled in wild 1, the most northern population, as hybrids. All of these admixed mink from the wild populations were partially assigned to the black phase, except for a mink from wild 1 that was partially assigned to brown.

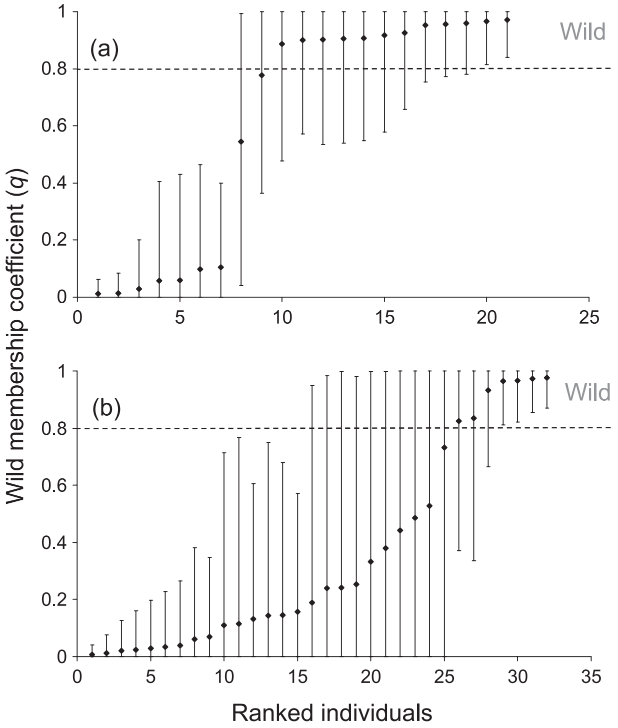

Fig. 3 Membership coefficients (*q*) ranked in increasing order for American mink (*Neovison vison*) sampled in the wild during 2005–2006 in Ontario, Canada. Included are 90% probability limits to the wild cluster from five replicate runs of $K = 4$ in Structure 2.2 for all individuals from mixed 1 (a) and mixed 2 (b) populations. A *q* of ≥ 0.80 is considered a 'wild' mink. Below the 80% threshold are both hybrid individuals ($q_{\text{wild}} + q_j + \ldots q_n \geq 0.80$) with a partial but not complete wild genotype and farm individuals with a $q \geq 0.80$ to one of the other clusters.

Pairwise F_{ST} values were estimated for the $K = 4$ groupings plus a separate group created for the hybrid individuals. All populations were significantly differentiated ($P < 0.05$) after 1000 permutations (Table 4). The wild mink population, which was not differentiated by the two geographical groupings (pairwise $F_{ST} = 0.027$), demonstrated moderate to high differentiation from the domestic mink populations, with the greatest difference being observed with the iris population. Iris mink displayed the greatest differentiation from all other populations (Table 4). The brown and black domestic mink were moderately differentiated. The hybrid

Table 4 Pairwise F_{ST} for the four populations of American mink (*Neovison vison*) sampled in the wild and in mink farms during 2005–2006 in Ontario, Canada. Populations were assigned using Structure 2.2 (Pritchard *et al.* 2000) and the wild-caught hybrids that assigned to more than one population cluster (the mink for the mahogany colour phase line were excluded as they assigned as farm-brown/black hybrids), of which all were statistically significant ($P < 0.05$) after 1000 permutations

	Hybrid	Farm – Brown	Farm – Black	Farm – Iris
Wild	0.0233	0.0918	0.1158	0.1738
Hybrid		0.0591	0.0575	0.1537
Farm – Brown			0.0617	0.1571
Farm – Black				0.1691

Table 5 Mean factor scores plus lower (LCL) and upper (UCL) 95% confidence intervals for the first three components in a principal components analysis of allele frequencies of mink assigned to domestic, wild, and hybrid ancestries based on Bayesian assignment. Mink were sampled in Ontario, Canada during 2005 and 2006

Group	*N*	Factor 1	LCL 1	UCL 1	Factor 2	LCL 2	UCL 2	Factor 3	LCL 3	UCL 3
Domestic	105	–0.519	–0.692	–0.345	–0.545	–0.747	–0.343	0.145	–0.064	0.354
Wild	75	0.748	0.588	0.907	0.717	0.603	0.831	0.005	–0.202	0.212
Hybrid	35	–0.047	–0.338	0.244	0.100	–0.108	0.307	–0.446	–0.730	–0.160

group displayed low to moderate differentiation from all populations except from the iris population, which only contributed to the genotype of one of the hybrids in each of mixed 1 and mixed 2 (Fig. 2).

Supporting analyses

Our simulated mink of known origin had mean (± 95% confidence interval) wild membership coefficients of 0.975 (± 0.005) for the 100 wild mink, 0.522 (± 0.077) for hybrids, and 0.036 (± 0.009) for the domestic mink. When we assigned these simulated mink to the three groups (wild, hybrid, and domestic) according to our criterion of $q \geq 0.80$, we had an overall rate of correct classification of 96.2%. We were able to correctly identify 100% of wild mink, 98% of domestic mink (two identified as hybrid) and 83% of hybrid mink (8.5% misassigned to each of domestic and wild groups). We could have improved our overall correct classification rate to 96.6% by shifting to a criterion of $q \geq 0.90$, where we would have correctly identified 97% of wild mink (3% called hybrids), 95% of domestic mink (5% called hybrids), and 94% of hybrid mink (3% misassigned to each of domestic and wild groups). However, given our hypothesis, that domestic mink are escaping and hybridizing with wild mink, we preferred to err on the side of being conservative in identifying hybrids. Thus, we chose to use the criterion of $q \geq 0.80$.

The first three components in our PCA had eigenvalues of 0.292, 0.275, and 0.214, respectively. Because each individual mink had a factor score for each principal component, we could estimate the mean (± 95% confidence intervals) factor scores for each of the three groups from our $K = 2$ analysis (wild, hybrid, and domestic). These confidence intervals did not overlap for the first two principal components (Fig. 4; Table 5), supporting our Structure-based assignments. Bivariate plots of the component loadings

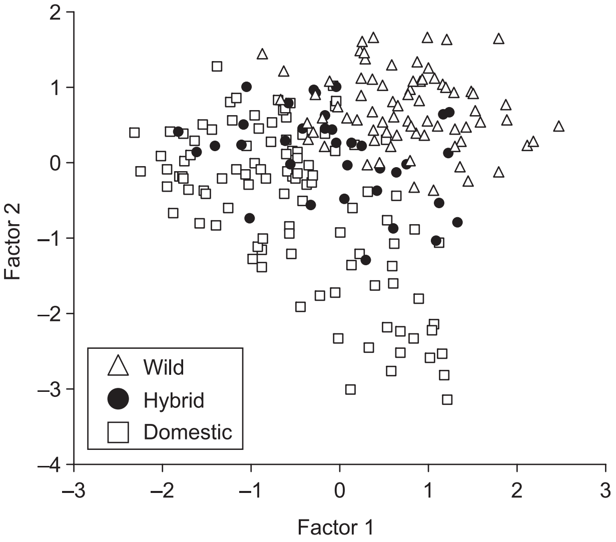

Fig. 4 Plot from principal components analysis (PCA) of allele frequencies showing individual mink (*Neovison vison*) sampled in Ontario that were classified in a Bayesian assignment test as domestic, wild, or domestic–wild hybrid. Factor scores are shown for individual mink on eigenvector 1 (*X* axis) and 2 (*Y* axis).

(not shown) demonstrated that component 1 largely described differences between black or brown domestic mink and wild mink at one locus (Mvi 4001). Component 2 described differences between iris and wild mink at Mvi 1014, where iris mink were strongly differentiated based on one allele. The third component, which had overlapping factor scores for the assigned groups (Table 5), mostly described differences within the pelt colour lines, rather than differences between domestic and wild mink.

Discussion

Our results support the hypothesis that domestic mink are escaping and persisting within wild mink populations. Furthermore, we found strong evidence that these feral mink are hybridizing with wild mink. The presence of domestic mink in the natural environment and the evidence of their interbreeding with wild conspecifics are of serious concern. Possible genetic consequences of these introductions may include reduced fitness and disruption of local adaptation via the introduction of maladaptive gene complexes (Rhymer & Simberloff 1996; Allendorf *et al*. 2001; McGinnity *et al*. 2003; Hutchings & Fraser 2008). Work on salmonids has shown an increased risk to the persistence of native populations with repeat introductions (McGinnity *et al*. 2003). In the case of mink, the kind of chronic escapement from ranches suggested by Bowman *et al*. (2007) could have a similarly depressing effect. Introgressive hybridization of wild populations with domesticated animals may contribute to genetic homogenization, disrupt population structure, and contribute to local extinctions by the disruption of local adaptations (Rhymer & Simberloff 1996; Allendorf *et al*. 2001; Randi 2008). Therefore, domestic–wild mink hybrids may be a threat to natural American mink populations. The potential threat is magnified owing to the geographical extent of the problem. Although this study occurred over a small spatial scale, mink ranches occur across much of the native range of mink (Joergensen 1985), meaning that hybridization could be widespread. It appears that more extensive genetic analysis and studies to assess the fitness consequences of hybridization are warranted.

The overall proportion of domestic and hybrid mink (64%) was more than wild mink caught in mixed 1 and mixed 2. Of the individuals sampled from mixed 1, only 57% were assigned as wild and even more dramatically, only 22% of the mink sampled in mixed 2 were wild animals. Thus, a majority of the mink sampled in close proximity to mink farms appeared to be either farm escapees or descendants of escapees. Moreover, we estimated that at least 28% of mink in these putatively mixed populations were domestic–wild hybrids or hybrid backcrosses. Although we have not attempted to identify backcrosses per se, it appears that backcrosses in both directions were prevalent in the mixed 2 population, but much less so in mixed 1 (Fig. 3). This suggests that the landscape around mixed 2 is more affected by longer-term problems with escapement. The presence of backcrossed individuals and such complex composition of hybrids suggests that domestic alleles are being introgressed into the wild mink population, which may be a concern for the future sustainability of wild mink.

The heterozygote deficiency observed in some domestic mink colour phases was expected because of line-breeding practices (Joergensen 1985; Belliveau *et al*. 1999). However, we found high levels of genetic variability in both brown and black domestic mink. Belliveau *et al*. (1999) suggested that this variability could result from particular breeding practices on farms, or from the increased reproductive performance that has been observed in brown mink relative to other colour phases, which may result in less selective pressure and drift than other colour phases (Joergensen 1985; Dunstone 1993; Belliveau *et al*. 1999). We suggest that the apparent deficit of heterozygotes in the mixed populations may be a Wahlund effect. Such an effect would result from overlapping individuals from different populations, such as the domestic mink and wild mink in mixed 1 and mixed 2. The same may be the case for wild 1 where five hybrids were identified (Weir & Cockerham 1984).

We observed genetic differentiation between the wild, hybrid, and domestic populations. Hybrids were moderately differentiated from the populations that had contributed most to their blended genotypes, whereas they were highly differentiated from the iris population. The iris population displayed the greatest divergence from all other populations and the most drift from a shared common ancestor. Given their specialized colour and the intensive assortative mating required to maintain it, this is not surprising (Joergensen 1985). However, not only were the iris mink the most distinct genetically, but they only contributed to the genotypes of 2 of the 21 hybrids. Moreover, out of 19 domestic mink identified in the mixed populations, only one was iris. Iris mink are relatively rare in ranches, and therefore we expect that relatively few mink of this colour phase escape. Furthermore, their rarity in the wild may be related to lower *in situ* survival or reproductive success. For example, it is well known that light coloured mink are highly susceptible to Aleutian Disease virus (Ellis 1996).

Conclusions and future directions

Introgressive hybridization has been shown to be a threat to recovery efforts for several species including the rock partridge in Greece (up to 20% admixed; Barilani *et al*. 2007); the grey wolf (Italy 5% admixed; Randi & Lucchini 2002; Verardi *et al*. 2006); and the wildcat in Bulgaria (17% admixed), Belgium (5% admixed), Portugal (14%), Italy (8% admixed), Hungary (31% admixed), and Scotland (41% admixed; Randi *et al*. 2001; Pierpaoli *et al*. 2003; Lecis *et al*. 2006). Management recommendations have been made based in part on the observed proportion of admixture in these various populations. The southern Ontario mink examined in our study displayed comparatively large amounts of admixture of at least 28%.

Bowman *et al*. (2007) provided evidence of declining mink populations in Canada despite the apparent supplementation of wild populations by domestic mink. We have confirmed that wild populations are indeed being supplemented by domestic animals. Moreover, we identified several hybrids in regions thought to be distant from mink farming, which illustrated the far-reaching effects of these introductions. Our findings are consistent with the suggestion of Bowman *et al*. (2007) that instead of increasing abundance, escapees may be directly linked to declines in wild mink populations. There are two avenues by which population declines of wild mink may be induced by the mink escaping from mink farms. First, as we have shown, introgressive hybridization with wild mink can occur. This may lead to the introduction of maladaptive genes into the natural mink population, or the disruption of locally adapted gene complexes (Rhymer & Simberloff 1996; Allendorf *et al*. 2001; Randi 2008). Second, diseases such as Aleutian disease, a highly infectious and often fatal parvovirus found in many mink farms (Bloom *et al*. 1980) may be introduced into natural mink populations via contact with domestic mink. Our study only demonstrates that the potential for these two avenues exists; further research will be required to demonstrate fitness reductions in wild mink related to introgression or disease.

In light of the potential for these effects, mink farmers and relevant government agencies should take steps to mitigate the impact of mink farms on adjacent natural ecosystems. A policy enforcing a minimum standard of biosecurity both preventing human entrance and mink exit would appear to be important. In Ontario, a licensing mechanism for mink farms (and those with other domesticated wildlife) may be required to facilitate improved biosecurity. Eradication and control programmes in Estonia, Belarus, Finland and portions of the UK have shown success in curbing or eliminating their feral mink problem (Bonesi & Palazon 2007). Hunting programmes in Iceland and Lithuania have been less successful. In fact, in Iceland the feral mink population appears to have increased (Bonesi & Palazon 2007). Unfortunately, whereas these programmes may provide some guidance for dealing with a feral mink problem, the issue is much more complex within the endemic range of the American mink. For example, eradication programmes would have to be carefully targeted to avoid wild mink, but this would be complicated by the level of admixture observed in our study. Ideas for managing this hybridization and introgression could be taken from work carried out with managing red wolf hybridization with coyotes (Adams *et al*. 2007) as well as from the projects for feral American mink eradication programmes in Europe summarized by Bonesi & Palazon (2007).

An overriding trend in domestic–wild hybrid populations of many species is that wild populations are first depleted by anthropogenic activities. Subsequent recovery efforts are then hampered as the genetic integrity of the remaining wild populations is compromised by anthropogenic-induced introgression (Allendorf *et al*. 2001; Randi 2008). We have found that the wild American mink populations are likewise being genetically compromised by anthropogenically induced hybridization and introgression with greater rates than has been observed in many similar studies. Therefore, we suggest that there is an urgent need for addressing this issue if we are to preserve the genetic integrity of our native mink populations.

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